2161-Plat
A Novel Photosynthetic Strategy for Adaptation to Low Iron Environments
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Iron availability is a major limiting factor for photosynthesis and hence for life in most of the aquatic environments on earth. . Cyanobacteria are important primary producers and prevail over Fe- deficiency by de-repressing the *isiAB* operon, which codes for the antenna protein IsiA and flavodoxin. We demonstrate that under nanomolar iron concentrations, a giant IsiA-Photosystem I supercomplex is formed, consisting of a Photosystem I trimer encircled by two complete IsiA rings with 18 and 25 copies in the inner ring and outer ring, respectively. The IsiA-Photosystem I supercomplex contains more than 850 chlorophylls and has a mass of 3.2 MDa, making it the most complex membrane protein that has been isolated to date. Ultrafast fluorescence spectroscopic results show fast and efficient excitation transfer and trapping in the supercomplex. The electron transfer throughput of Photosystem I is increased by 300%, an evolutionary adaptation that has allowed cyanobacteria to avoid oxidative stress. This adaptation of the photosynthetic apparatus confers an enormous, as-yet unrecognized, evolutionary advantage to cyanobacteria living under conditions of severe Fe stress and thereby have adapted to the modern low iron concentration in aquatic environments.
1. Eldridge, M. et al. Aquatic Microbial Ecology 35, 79-91 (2004).
2. Martin, J. & Fitzwater, S. Nature 331, 341-343 (1988).
3. Wilhelm, S. Aquatic Microbial Ecology 9, 295-303 (1995).
4. Bibby, T. S., Nield, J. & Barber, J. Nature 412, 743-5 (2001).
5. Boekema, E. J. et al. Nature 412, 745-8 (2001).
6. Singh, A., McIntyre, L. & Sherman, L. Plant Physiology 132, 1825 (2003).

Platform AH: Membrane Receptors & Signal Transduction

2162-Plat
A Novel Method to Probe Membrane Protein Topology Using Unnatural Amino Acid Mutagenesis and Antibody Epitope Tagging
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We have developed a novel strategy to probe the topology of membrane proteins in their native bilayer environment. Our technique relies on unnatural amino acid mutagenesis to incorporate *p*-azido-L-phenylalanine at a specific site in the expressed target protein. The reactive azido moiety facilitates Staudinger-Bertozzi ligation chemistry to introduce a monoclonal antibody (mAb) epitope-tagged phosphine derivative. This site-specific labeling method allows the flexibility and precision of single codon scanning, and appears to be superior to current biochemical approaches that rely on chemical modifications and/or introduction of epitope tags by mutagenesis. Our approach can be used to identify gross topological determinants for transmembrane proteins of unknown topology as well as to elucidate secondary structural elements with chemical precision. We demonstrate the experimental feasibility of our technique on human C-C chemokine receptor 5 (CCR5), a heptahelical transmembrane G protein-coupled receptor (GPCR) of known topology. CCR5 is a major co-receptor for HIV-1 entry into host cells. We labeled CCR5 in membranes with FLAG peptide epitope-phosphine at various sites on the receptor's intra- and extracellular surfaces. The differential reactivity of the FLAG epitope-phosphine reagent for the azido group on the various CCR5 mutants correlated to the known topology of CCR5 and defined specific helical boundaries. We further applied the new label/probe technology to mammalian cells in culture in order to label extracellular sites on surface-expressed CCR5. Our new method appears to be satisfactory to probe membrane protein topology in polytopic membrane proteins and has potential applications in the study of receptor signaling mechanisms in live cells.

2163-Plat
Large-Scale MD Simulations Reveal Structural Elements of the Activated State in the 5-HT2a Receptor and their Relation to Cholesterol Dynamics
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The 5-HT_{2A} Serotonin receptor (5-HT_{2A}R) is a G-protein coupled receptor (GPCR) targeted by therapeutic drugs as well as hallucinogens such as LSD. We have previously shown that different modes of receptor activation and distinct cellular signaling are produced by hallucinogens and nonhallucinogenic congeners acting on the 5-HT_{2A}R (Weinstein, 2006). We characterize the ligand-dependent states of 5-HT_{2A}R from MD simulations of an experimentally-validated homology model based on rhodopsin and β_2AR structures complemented by cognate information about other GPCRs. At different stages of 0.3 μs simulations of 5-HT-bound 5-HT_{2A}R we observe sequential conformational changes that produce structural characteristics of receptor activation: (*1*) the extracellular part of TM6 moves inwards; (*2*) the toggle switch W6.48 flips to become parallel to the membrane; (*3*) the intracellular part of TM6 moves outwards and (*4*) the ionic lock in the DRY motif breaks. In the parallel simulations of 5-HT_{2A}R with LSD (currently at 0.12 μs), only some of the same active-like components are observed (e.g., TM7 moving away from TM2, and the breaking of the ionic lock). Comparing the dynamics of 5-HT_{2A}R complexed with LSD and 5-HT, we found that H8 of the LSD-bound receptor undergoes less rearrangement during the same time period, and that the interaction of extracellular loop 2 with LSD is stronger than with 5-HT, but that local distortions in TMs (e.g., proline kinks) are similar in the two systems. Interestingly, correlation analysis indicates that some of the local changes in 5-HT_{2A}R relate to the dynamics of cholesterol in the membrane, at a series of preferred sites of interaction with the receptor, in a manner similar to those reported previously from μs rhodopsin simulations (Grossfield et al., 2006; Khelashvili et al., 2009).

2164-Plat
Molecular Model of the Opsin-G-Protein Complex
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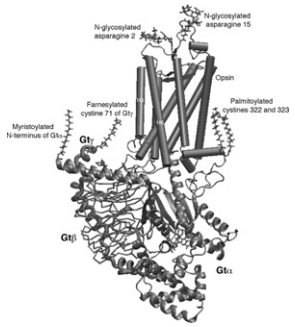
A recent highlight in the structural biology of G-protein-coupled receptors includes the structural elucidation of opsin in an active-state conformation (Ops*). We modeled the complex of opsin with the heterotrimeric G-protein transducin (Ops*-$G_t\alpha\beta\gamma$, figure below), using the NMR structure of an 11-residues peptide from the C-terminus of the alpha-subunit of transducin ($G_t\alpha$CT) and the crystal structures of opsin in its G-protein interacting conformation (Ops*-$G_t\alpha CT_{K341L}$), of rhodopsin in an inactive state, and of transducin ($G_t\alpha\beta\gamma$•GDP). We reconstructed the C-terminal α5 helix of $G_t\alpha$ and docked it to the open binding site in Ops*. It has been proposed that a 40°-tilt of $G_t\alpha\beta\gamma$ relative to the α5 helix is necessary to avoid steric clashes between $G_t\beta\gamma$ and the membrane. We propose an alternative model without these massive changes in α5 helix packing. With an alternative conformation of the intra-cellular loop connecting helix 5 and helix 6 in Ops* it is possible to obtain a model that maintains the Ops*-induced α_L-type C-capping conformation of Gt_αCT and its key interactions with Ops*. The model will be used for detailed MD simulations of the complex in the membrane and to design cross-linking experiments for biochemical validation.



2165-Plat
Insights into G-Protein Coupled Receptors Activation from All-Atom Molecular Dynamics Simulations
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G protein coupled receptors (GPCRs) are a large family of integral membrane proteins involved in many signal transduction pathways. The recently crystallized structures of two engineered adrenergic receptors (ARs)[1,2] and of ligand-free opsin bound to a G-protein peptide[3] have opened new avenues for the understanding of the molecular mechanisms of action of GPCRs, but they also generated some controversy on the proposed mechanism of GPCR activation.
To understand the molecular details of GPCR activation, we carried out submicrosecond molecular dynamics simulations of wild type β1AR and β2AR and of rhodopsin dimer in explicit lipid bilayer under physiological conditions.
Our simulations showed that the equilibrated structures of ARs recover all the previously suggested features of inactive GPCRs, including formation of a crucial salt bridge between the cytoplasmatic moieties of helices III and VI (''ionic-lock'') that is absent in the crystal structures[4].
We found that cooperation between a number of highly conserved residues is a key component in the early steps of the activation mechanism of diffusible

ligand class A GPCRs and that ''ionic-lock'' formation in ARs is directly correlated with the protonation state of a highly conserved aspartic acid, even though the two sites are located more than 20Å away from each other[5].
Additionally, following the real time evolution of the rhodopsin dimer up to microseconds after photoexcitation, we propose a tandem mechanism for signal transduction where one monomer is responsible for light detection while the other one serves as G-protein coupling site[6]. This interface-mediated pathway suggests oligomerization-aided signal transduction as a crucial biological mechanism to enhance activation of GPCRs.
[1] Cherezov et al, Science, 2007.
[2] Warne et al, Nature, 2008.
[3] Scheerer et al. Nature, 2008.
[4] Vanni et al. Biochemistry, 2009.
[5] Vanni et al. Submitted.
[6] Neri et al. Submitted.

2166-Plat
Dynamics of Gq Protein Interactions with PLCβ3 Studied by TIRF Microscopy
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G protein-mediated activation of phospholipase Cβ (PLCβ) represents a primary mechanism to regulate many physiological events such induce smooth muscle contraction, secretion and modulation of synaptic transmission. Both Gαq- and Gβγ-subunits are known to interact and activate PLCβ enzymes, however little is known about the dynamics of this interactions and the relative contribution of the G protein subunits in intact cells. Using fluorescence resonance energy transfer- (FRET-) based assays in single intact cells we studies kinetics of receptor-induced interactions between Gβγ- and Gαq-subunits, interactions of both Gαq and Gβγ with PLCβ3 as well as interactions of regulator of G proteins signalling 2 (RGS2) with Gαq- and Gβγ-subunits. In order to restrict the protein/protein interaction studies to the cell membrane we applied total internal reflection (TIRF) microscopy. High temporal resolution ratiometric FRET imaging uncovered a markedly faster dissociation of Gαq and PLC upon withdrawal of purinergic agonists compared to the deactivation of Gq proteins in the absence of PLCβ3. This apparent difference in kinetics could be contributed to the GTPase-activating property of PLCβ3 in living cells. Furthermore we found that PLCβ3 modulated Gq protein kinetics to a similar extent compared to RGS2, which in vitro is about 100 fold more efficient in activating Gq-GTPase activity. We observed that both Gαq subunits and Gq-derived Gβγ-subunits interact with PLCβ3 in response to receptor stimulation. In the absence of receptor stimulation we did neither detect any specific FRET signals between Gq protein subunits and PLCβ3 nor did we detect any interactions between RGS2 and Gαq subunits. Finally we could not detect agonist- dependent FRET between RGS2 and Gβγ-subunits. Taken together, ratiometric FRET-imaging under conditions of TIRF allowed new insights into dynamics and interaction patterns within the Gq signalling pathway.

2167-Plat
Role of Plasma Membrane Structuring on Interferon Receptor Assembly & Signaling
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Signaling in cells is mediated through multi-protein complexes, and is triggered by recognition of a chemical molecule, the ligand, to membrane receptors at the extracellular side. Binding in turn leads to activation of intracellular signaling. Understanding of the dynamical behavior of receptors and its nanometric organization in the membrane is fundamental to understand the processes of cell signaling. We are studying type I interferon (IFN) receptor, a member of the cytokine family, which plays a key role in early innate and adaptive immune responses upon infection by pathogens. It is puzzling how different members of the ligand type-1 IFN family elicit differential responses while binding to only one surface receptor. The latter comprises of two proteins ifnar1 and ifnar2, present at very low surface concentration of 100-1000 molecules/cell. Upon ligand binding a ternary complex is formed and downstream signaling pathways activated. Cells quickly and effectively respond, despite the very low amount of receptor present. Using single-molecule wide-field fluorescence microscopy we follow individual receptor in the plasma membrane of living HeLa cells. Receptor subunits were labeled post-translationally with synthetic dyes, or with fluorescent proteins. Each of the receptor subunit was transfected and expressed, separately and simultaneously, allowing measurements on single component as well as on the ternary complex formed upon IFN binding. Through correlative analysis of Ifnar1 and Ifnar2 mobility we visualized association/dissociation events of the ternary complex upon stimulation, and obtained information on receptor's diffusion constants. We observed a switching in between fast and slow motility and vice versa, and a confinement of receptor components in domains. Upon stimulation an increase of cross-correlation between the components was observed as well as a change in mobility. Our findings suggest a role for membrane nanostructure as platform for differential cell signaling.

2168-Plat
Involvement of Transmembrane Helix Dimerization and Rotation in Signaling by the Thrombopoietin Receptor
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In this study, we investigate the role of the transmembrane (TM) domain in the activation of one member of the cytokine receptor family: the thrombopoietin receptor (TpoR). The TM domain is thought to play a key role in the activation by facilitating receptor homodimerization and by transmitting the ligand-induced re-orientation of the extracellular domain to the cytoplasmic domain through conformational changes. However, the precise mechanisms underlying these events are not fully understood. Here, we considered several unanswered questions: Is the homodimerization of TpoR TM domain important in receptor activation? Which TM residues are involved in stabilizing receptor interactions? Additionally, we investigated the role of a constitutively activating mutation (S505N) and the mechanism of action of a piperidine-4-carboxylic acid TpoR agonist (Compound 2). We show that the TM domain of the human TpoR dimerizes strongly and that the full-length receptors exist as homodimers on the surface of mammalian cells in the absence of ligand. Our results indicate that TpoR can adopt two different conformations involving two different sets of residues. One of the contact interfaces mimics an inactive unliganded TpoR dimer and the other corresponds to an active conformation that is compatible with the constitutive signaling induced by the S505N mutation. We also show that Compound 2 interacts specifically with the N-terminus portion of the active dimer conformation. Overall, our results give new insight into the role of the TM domain in the activation mechanism of TpoR and provide a more detailed model of cytokine receptor activation and modulation by small molecule agonists.

2169-Plat
Spatio-Temporal Patterns of Reactive Oxygen Species Production in PDGF Signaling Revealed by Nanoparticle Imaging in Living Cells
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Signaling by PDGF (Platelet Derived Growth Factor) is involved in cell migration, for metastasis formation or reparation of vascular lesions. Hydrogen peroxide (H2O2) is a known second messenger in this pathway and its intracellular concentration regulates the cell response. However, conventional methods are unable to measure quantitatively its temporal evolution. Here, we propose a new approach based on the imaging of YVO4:Eu nanoparticles. Their luminescence is indeed modulated by the oxidation state of doping europium ions. After photoinduced reduction, their chemical oxidation by H2O2 can be monitored by the nanoparticle imaging. We demonstrated in vitro that these particles are efficient probes to dynamically and quantitatively measure H2O2 concentration. By internalizing these nanoparticles in mamallian cells, we measured the oxidant response to a PDGF stimulation. We revealed the temporal pattern of H2O2 production and determined the effective affinity of PDGF receptors for their ligand. We thus proved that a persistent stimulation was necessary to trigger a significant H2O2 production: this intrinsic filtering could be of major physiological interest for understanding reliable cell migration. This response implies transactivation of EGF receptors, which we proved to be dominant at short times. The comparison of normal and tumoral cells revealed a faster and more important H2O2 production in tumoral cells. This likely relies on the different expression levels of proteins of the signaling cascade and points to the potential role of signal transduction dynamics for the regulation of metastasis formation.
This work proposes the first quantitative measurements of the oxidant signaling in cells by the use of new nanoprobes. It more generally opens new perspectives for the study of the spatio-temporal organization of the cell response and its physiological importance.